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Request for grant of a

Patent

Form 1/77

Patents Act 1977

**① Title of invention**

- 1 Please give the title of the invention Novel Compounds

**② Applicant's details**

- ☐ First or only applicant

- 2a If you are applying as a corporate body please give:  
Corporate Name SMITHKLINE BEECHAM PLC

Country (and State of incorporation, if appropriate) UNITED KINGDOM

- 2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

**2c In all cases, please give the following details:**

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☐ **Second applicant (if any)**

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3a Have you appointed an agent to deal with your application?

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No ☐ go to 3b



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Agent's name

**Connell A C**

Agent's address:

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SMITHKLINE BEECHAM PLC  
SB HOUSE  
GREAT WEST ROAD  
BRENTFORD  
MIDDLESEX

Postcode

**TW8 9BD**

Agent's ADP  
number

6051676002

3b: If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name:

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P31574

4. Agent's or applicant's reference number *(if applicable)*

### Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Yes ☐

No   go to 6



***please give details below***

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☐ filing date (day month year)

☐ and the Section of the Patents Act 1977 under which you are claiming:

15(4) (Divisional) ☐      8(3) ☐      12(6) ☐      37(4) ☐

### ⑥ Declaration of priority

6. If you are declaring priority from previous application(s), please give:

Country of Filing

Priority application number  
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**6** *If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number*

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7

- applicant is not an inventor
- there is an inventor who is not
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- any applicant is a corporate body.

8 Please supply duplicates of claim(s), abstract, description and drawings).

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9 You or your appointed agent (see Rule 90 of the Patents Rules 1990) must sign this request.

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## 7 Inventorship

7. Are you (the applicant or applicants) the sole inventor or the joint inventors?

Please mark correct box

Yes ☐ No ☒

A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).)

## 8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77

Claim(s) 1

Description 32

Abstract

Drawing(s)

(NO FIGS  
22 PRESENT)

8b Which of the following documents also accompanies the application?

Priority documents (please state how many)

Translation(s) of Priority documents (please state how many)

Patents Form 7/77 - Statement of Inventorship and Right to Grant

Patents Form 9/77 - Preliminary Examination Report

Patents Form 10/77 - Request for Substantive Examination

## 9 Request

I/We request the grant of a patent on the basis of this application.

Signed A C Connell  
A C Connell

Date: 26 July 1996  
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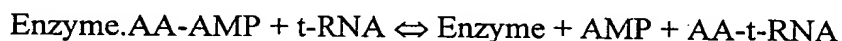
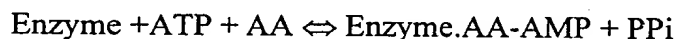
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## Novel Compounds

This invention relates to a newly identified t-RNA synthetase isolated from a *S.aureus* organism, polynucleotides encoding for such a synthetase, the use of such synthetase and polynucleotides, as well as the production of such synthetase and polynucleotides and recombinant host cells transformed with the polynucleotides.

t-RNA synthetases have a primary role in protein synthesis according to the following scheme:



in which AA is an amino acid.

Inhibition of this process leads to a reduction in the levels of charged t-RNA and this triggers a cascade of responses known as the stringent response, the result of which is the induction of a state of dormancy in the organism. As such selective inhibitors of bacterial t-RNA synthetase have potential as antibacterial agents. One example of such is mupirocin which is a selective inhibitor of isoleucyl t-RNA synthetase. Other t-RNA synthetases are now being examined as possible anti-bacterial targets, this process being greatly assisted by the isolation of the synthetase.

Isoleucyl t-RNA synthetase, isolated from *Staphylococcus aureus*, has already been described (Chalker A F, Ward J M, Fosberry A P and Hodgson J E, 1994, Gene 141:103-108).

The present invention relates to a polypeptide isolated from *S.aureus* WCUH29, characterised in that it comprises an amino acid sequence selected from SEQ ID NO 1A, SEQ ID NO 1B, SEQ ID NO 1C, SEQ ID NO 1D, SEQ ID NO 1E SEQ ID NO 1F, SEQ ID NO 1G and SEQ ID NO 1H, or a fragment, analogue or derivative thereof. The polypeptides are believed to be fragments of the enzymes phenylalanyl t-RNA synthetase (FRS) (SEQ ID NO 1A and SEQ ID NO 1B), prolyl t-RNA synthetase (PRS), histidyl t-RNA synthetase (HRS), methionyl t-RNA synthetase (MRS), valyl t-RNA synthetase (VRS), asparaginyl t-RNA synthetase (NRS), and arginyl t-RNA synthetase (RRS), respectively.

*S.aureus* WCUH29 has been deposited at the National Collection of Industrial and Marine Bacteria Ltd. (NCIMB), Aberdeen, Scotland under number NCIMB 40771 on 11 September 1995.

The invention also relates to the polypeptide having the amino acid sequence selected from SEQ ID NO 1A, SEQ ID NO 1B, SEQ ID NO 1C, SEQ ID NO 1D, SEQ ID NO 1E, SEQ ID NO 1F, SEQ ID NO 1G and SEQ ID NO 1H or a derivative thereof.





Hereinafter the term polypeptide(s) will be used to refer to the tRNA synthetase *per se*, fragments, analogues and derivatives thereof.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

5 In particular the invention provides a polynucleotide having a DNA sequence selected from SEQ ID NO 2A, SEQ ID NO 2B, SEQ ID NO 2C, SEQ ID NO 2D, SEQ ID NO 2E, SEQ ID NO 2F, SEQ ID NO 2G and SEQ ID NO 2H. The invention further provides a polynucleotide encoding a polypeptide from *S.aureus* WCUH29 and characterised in that it comprises a DNA sequence selected from SEQ ID NO 2A, SEQ  
10 ID NO 2B, SEQ ID NO 2C, SEQ ID NO 2D, SEQ ID NO 2E, SEQ ID NO 2F, SEQ ID NO 2G and SEQ ID NO 2H.

The polynucleotides having the DNA sequence given in SEQ ID NO 2A to 2H were obtained from a library of clones of chromosomal DNA of *S.aureus* WCUH29 in *E.coli*.

15 To obtain the polynucleotide encoding the polypeptide using one of the DNA sequences SEQ ID NO 2A to 2H typically a library of clones of chromosomal DNA of *S.aureus* WCUH29 in *E.coli* or some other suitable host is probed with a radiolabelled oligonucleotide, preferably a 17mer or longer, derived from the partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using high  
20 stringency washes. By sequencing the individual clones thus identified with sequencing primers designed from the original sequence it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook, J. in  
25 MOLECULAR CLONING, A Laboratory Manual [2nd edition 1989 Cold Spring Harbor Laboratory. see Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70].

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The  
30 DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence shown in SEQ ID NO 2A to 2H or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide.

35 The present invention includes variants of the hereinabove described polynucleotides which encode fragments, analogs and derivatives of the polypeptide characterised by a deduced amino acid sequence selected from SEQ ID NO 1A to 1H.

The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same polypeptide characterised by a deduced amino acid sequence selected from any one of sequences SEQ ID NO 1A to 1H as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

A polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence characterised by a DNA sequence selected from any one of SEQ ID NO 2A to 2H. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotide which encodes for the mature polypeptide, i.e. the native methionyl tRNA synthetase, may include only the coding sequence for the mature polypeptide or the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention therefore includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence at either the 5' or 3' terminus of the gene which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by the pQE series of vectors (supplied commercially

by Quiagen Inc.) to provide for purification of the polypeptide fused to the marker in the case of a bacterial host.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably at least 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the polypeptide characterised by a deduced amino acid sequence selected from any one of sequences SEQ ID NO 1A to 1H.

The deposit referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited material, and no such license is hereby granted.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide characterised by a deduced amino acid sequence SEQ ID NO 1A to 1H, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide characterised by a deduced amino acid sequence SEQ ID NO 1A to 1H may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence

which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

5 The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the  
10 coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

15 The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is therefore provided a process for producing the polypeptide of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host and  
20 recovering the expressed product. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a cosmid, a  
25 phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

30 Suitable expression vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA. However, any other vector may be used as long as it is replicable and viable in the host.

35 The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli. lac* or *trp*, the phage lambda P<sub>L</sub> promoter and other promoters known to control expression of genes in eukaryotic or prokaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. The polypeptides of the present invention can be expressed using, for example, the *E. coli* *tac* promoter or the protein A gene (*spa*) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include *lacI*, *lacZ*, T3, T7, *gpt*, lambda P<sub>R</sub>, P<sub>L</sub> and *trp*. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA

polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the coding sequences may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pET-3 vectors (Stratagene), pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pBlueBacIII (Invitrogen), pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage  $\lambda$  (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), a baculovirus insect cell system, , YCp19 (*Saccharomyces*). See, generally, "DNA Cloning": Vols. I & II, Glover *et al.* ed. IRL Press Oxford (1985) (1987) and; T. Maniatis *et al.* ("Molecular Cloning" Cold Spring Harbor Laboratory (1982).

In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal.

Polypeptides can be expressed in host cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Depending on the expression system and host selected, the polypeptide of the present invention may be produced by growing host cells transformed by an expression vector described above under conditions whereby the polypeptide of interest is expressed. The polypeptide is then isolated from the host cells and purified. If the expression system secretes the polypeptide into growth media, the polypeptide can be purified directly from the media. If the polypeptide is not secreted, it is isolated from cell lysates or recovered from the cell membrane fraction. Where the polypeptide is localized to the cell surface, whole cells or isolated membranes can be used as an assayable source of the desired gene product. Polypeptide expressed in bacterial hosts such as *E. coli* may require isolation from inclusion bodies and refolding. Where the mature protein has a very hydrophobic region (normally at the C-terminus) which leads to an insoluble product of

overexpression, it may be desirable to express a truncated protein in which the hydrophobic region has been deleted. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

5 The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid  
10 chromatography (HPLC) can be employed for final purification steps.

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

15 In a further aspect, the present invention further comprises a protein obtainable by expressing the polynucleotide having the DNA sequence selected from SEQ ID NO 2A to 2H.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

20 A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "double-stranded DNA molecule" refers to the polymeric form of deoxyribonucleotides (bases adenine, guanine, thymine, or cytosine) in a double-stranded  
25 helix, both relaxed and supercoiled. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according  
30 to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having the sequence homologous to the mRNA).

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when  
35 placed under the control of appropriate regulatory sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding



sequence. For purposes of defining the present invention, the promoter sequence is bound at the 3' terminus by a translation start codon (e.g., ATG) of a coding sequence and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the expression (i.e., the transcription and translation) of a coding sequence in a host cell.

A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature.

In accordance with another aspect of the present invention, there are provided inhibitors to such polypeptides which are useful as antibacterial agents.



This invention also provides a pharmaceutical or veterinary composition which comprises an inhibitor of the present invention (hereinafter referred to as the 'drug') together with a pharmaceutically or veterinarily acceptable carrier or excipient. The compositions may be formulated for administration by any route, and would depend on the disease being treated. The compositions may be in the form of, for instance, tablets, capsules, powders, granules, suppositories, lozenges and liquid or gel preparations, including oral, topical and sterile parenteral suspensions.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional formulations well known in the art, for example, as described in standard text books of pharmaceuticals and cosmetics, such as Harry's Cosmeticology, 7th edn, ed Wilkinson and Moore, 1982, George Godwin, Harlow, England and the British Pharmacopoeia.

Suppositories will contain conventional suppository bases, e.g. cocoa-butters or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the drug and a sterile vehicle. The drug, depending on the vehicle and concentration used, can be suspended in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability the composition can be frozen after filling into the vial and water removed under vacuum. The dry lyophilized powder is then sealed in the vial. The drug can be

sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the drug.

For topical application to the ear, the drug may be made up into a suspension in a suitable liquid carrier, such as water, glycerol, diluted ethanol, propylene glycol, polyethylene glycol or fixed oils. For topical application to the eye, the drug is formulated as a suspension in a suitable, sterile aqueous or non-aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edetate; preservatives including bactericidal and fungicidal agents, such as phenylmercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage employed for compositions administered topically will, of course, depend on the size of the area being treated. For the ears and eyes each dose will typically be in the range from 10 to 100 mg of the drug.

Veterinary compositions for intramammary treatment of mammary disorders in animals, especially bovine mastitis, will generally contain a suspension of the drug in an oily vehicle.

The compositions may contain from 0.1% to 99% by weight, preferably from 10-60% by weight, of the drug, depending on the method of administration. Where the compositions are in unit dose form, each dosage unit will preferably contain from 50-500 mg, of the drug. The dosage as employed for adult human treatment (average weight about 70 kg) will preferably range from 100 mg to 3 g per day, for instance 250 mg to 2 g of the drug per day, depending on the route and frequency of administration. Alternatively, the drug may be administered as part of the total dietary intake of a non-human animal. In this case the amount of drug employed may be less than 1% by weight of the diet and in preferably no more than 0.5% by weight. The diet for animals may consist of normal foodstuffs to which the drug may be added or the drug may be included in a premix for admixture with the foodstuff. A suitable method of administration of the drug to animals is to add it to the non-human animal's drinking water. In this case a concentration of the drug in the drinking water of about 5-500 mg/ml, for example 5-200 mg/ml, is suitable.

In a further aspect, this invention provides a method of screening drugs to identify those which interfere with the interaction of the methionyl tRNA synthetase. The enzyme mediated incorporation of radiolabelled amino acid into tRNA may be measured by the aminoacylation method which measures amino acid-tRNA as trichloroacetic acid-precipitable radioactivity from radiolabelled amino acid in the presence of tRNA and ATP (Hughes J, Mellows G and Soughton S, 1980, FEBS Letters, 122:322-324). Thus

inhibitors of methionyl tRNA synthetase can be detected by a reduction in the trichloroacetic acid precipitable radioactivity relative to the control. Alternatively the tRNA synthetase catalysed partial PPi/ATP exchange reaction which measures the formation of radiolabelled ATP from PPi can be used to detect methionyl tRNA synthetase inhibitors (Calender R & Berg P, 1966, Biochemistry, 5, 1681-1690).

In order to facilitate understanding of the following example certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case 'p' preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 µg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 µl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 µg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37 C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., *et al.*, *Id.*, p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

**Example 1****Isolation of DNA coding for a tRNA Synthetase from *S.aureus* WCUH29**

The polynucleotide having the DNA sequence given by one of SEQ ID NO 2A to 2H was obtained from a library of clones of chromosomal DNA of *S.aureus* WCUH29 in *E.coli*. Libraries may be prepared by routine methods, for example:

*Methods 1 and 2*

Total cellular DNA is isolated from *S.aureus* strain WCUH29 (NCIMB 40771) according to standard procedures and size-fractionated by either of two methods.

*Method 1.*

10 Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 11kbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI, the library packaged by standard procedures and *E.coli* infected with the packaged  
15 library. The library is amplified by standard procedures.

*Method 2.*

Total cellular DNA is partially hydrolsed with a combination of four restriction enzymes (RsaI, Pali, AluI and Bsh1235I) and size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector  
20 Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

**SEQ ID NO 1A - *S. aureus* Phenylalanyl tRNA Synthetase (- Alpha Sub-unit)**

1 MSEQQTMSEL KQQALVDINE ANDERALQEV KVKYLGKKGS VSGLMKLMKD  
 51 LPNEEKPAFG QKVNELRQTI QNELDERQQM LVKEKLNKQL AEETIDVSLP  
 101 GRHIEIGSKH PLTRTIEEIE DLFLGLGYEI VNGYEVEQDH YNFEMNLNPK  
 151 SHPARDMQDS FYITDEILLR THTSPVQART MESRHGQGPV KIICPGKVYR  
 201 RDSDDATHSH QFTQIEGLVV DKNVKMSDLK GTLELLAKKL FGADREIRLR  
 251 PSYFPFTEPS VEVDVSCFKC KGKGCNVCKH TGWIEILGAG MVHPNVLEMA  
 301 GFDSSEYSGF AFGMGPDRIA MLKYGIEDIR HFYTNDVRFL DQFKAVEDRG  
 351 DM

**SEQ ID NO 2A**

1 ATGTCTGAAC AACAAACAAT GTCAGAGTTA AAACAACAAG CGCTTGTAGA  
 51 TATTAATGAA GCAAATGATG AACGTGCACT GCAAGAAGTT AAAGTGAAAT  
 101 ACTTAGGTAA AAAAGGGTCA GTTAGCGGAC TAATGAAATT GATGAAGGAT  
 151 TTGCCGAATG AAGAGAAACC TGC GTTTGGT CAAAAAGTGA ATGAATTGCG  
 201 TCAAACAATT CAAAATGAAT TAGATGAAAG ACAACAGATG TTAGTTAAAG  
 251 AAAAATTAAA TAAGCAATTG GCTGAAGAAA CAATTGATGT ATCATTACCA  
 301 GGTGTCATA TTGAAATCGG TTCAAAGCAT CCATTAACAC GTACAATAGA  
 351 AGAAATTGAA GACTTATTCT TAGGTTTAGG TTATGAAATT GTGAATGGAT  
 401 ATGAAGTTGA ACAAGATCAT TATAACTTCG AAATGCTGAA TTACCTAAA  
 451 TCACACCCTG CACGTGATAT GCAAGATAGT TTCTATATTA CGGATGAAAT  
 501 TTTATTACGT ACGCATACAT CACCAGTGCA GGCACGTACG ATGGAATCAC  
 551 GTCATGGTCA AGGTCCAGTT AAAATTATTT GCCCTGGTAA AGTGTATCGT  
 601 CGTGA CTCTG ATGATGCGAC ACATAGTCAT CAATTTACAC AAATCGAAGG

651 ATTAGTTGTT GATAAAAACG TTAAAATGAG TGATTGAAA GGCACCTTAG  
 701 AATTGTTAGC TAAGAAATTA TTTGGTGCTG ATCGTGAAAT TCGTTTACGT  
 5 751 CCAAGTTACT TCCCATTAC TGAACCTTCT GTAGAAGTTG ATGTGTCATG  
 801 TTTTAAATGT AAAGGAAAAG GTTGTAATGT GTGTAAACAC ACAGGATGGA  
 851 TTGAAATTTT AGGTGCTGGA ATGGTACATC CTAATGTATT AGAAATGGCT  
 10 901 GGTTTTGATT CTTCAGAGTA CTCTGGATTG GCATTGTTGTA TGGGACCAGA  
 951 CCGTATTGCA ATGTTGAAAT ATGGTATAGA AGATATTCGT CATTTCTATA  
 15 1001 CTAATGATGT GAGATTTTGA GATCAATTTA AAGCGGTAGA AGATAGAGGT  
 1051 GACATG

**SEQ ID NO 1B - *S. aureus* Phenylalanyl tRNA Synthetase (- Beta Sub-unit)**

20 1 MLISNEWLKE YVTIDDSVSD LAERITRTGI EVDDLIDYTK DIKNLVVGFV  
 51 KSKEKHPDAD KLNVCQVDIG EDEPVQIVCG APNVDAGQYV IVAKVGGRLP  
 101 GGIKIKRAKL RGERSEGMIC SLQEIGISSN YIPKSFESGI YVFSQSQVPG  
 25 151 TDALQALYLD DQVMEFDLTP NRADALSMIG TAYEVAALYN TKMTKPETTS  
 201 NELELSANDE LTVTIENEDK VPHY SARVVH DVTIEPSPIW MQARLIKAGI  
 30 251 RPINNVVDIS NYVLLEYGQP LHMFDQDAIG SQQIVVRQAN EGKMTTLDD  
 301 TERELLTSDI VITNGQTPIA LAGVMGGDFS EVKEQTSNIV IEGAIFDPVS  
 35 351 IRHTSRRLNL RSESSSRFEK GIATEFVDEA VDRACYLLQT YANGKVLKDR  
 401 VSSGELGAFI TPIDITADKI NRTIGFDLSQ NDI VTIFNQL GFDTEINDDV  
 451 ITVLVPSRRK DITIKEDLIE EVARIYGYDD IPSTLPVFDK VTSGQLTDRQ  
 40 501 YKTRMVKEVL EGAGLDQAIT YSLVSKEDAT AFSMQQRQTI DLLMPMSEAH  
 551 ASLRQSLLPH LIEVASYNVA RKNKDVKLFE IGNVFFANGE GELPDQVEYL  
 601 SGILTGDYVV NQWQGGKETV DFYLAKGVVD RVSEKLNLEF SYRRADIDGL  
 45

651 HPGRTAEILL ENKVVGFIGE LHPTLAADND LKRTYVFELN FDALMAVSVG

701 YINYQPIPRF PGMSRDIALE VDQNIPAADL LSTIHAHGGN ILKDTLVFDV

5 751 YQGEHLEK GK KSI AIRLNYL DTEETLTDER VSKVQAEIEA ALIEQGA VIR

# SEQ ID NO 2B

10 1 ATGTTGATAT CAAATGAATG GTTGAAAGAA TATGTAACAA TCGATGATTC

51 TGTAAGTGAT TTGGCAGAAC GTATTACGCG CACAGGTATT GAAGTGGATG

101 ATTTAATTGA CTACACAAAA GATATCAAAA ATTTAGTTGT CGGTTTCGTT

15 151 AAGTCAAAAG AGAAACATCC TGATGCCGAT AAATTAAATG TTTGCCAAGT

201 TGATATCGGA GAAGACGAAC CTGTACAAAT CGTATGTGGT GCACCGAACG

20 251 TTGATGCAGG ACAATATGTC ATTGTTGCTA AAGTAGGTGG CAGATTGCCT

301 GGTGGTATTA AAATTAAGCG TGCCAAATTA CGCGGTGAAC GTTCAGAAGG

351 TATGATTTGT TCGTTACAAG AAATTGGTAT TTCAAGTAAC TATATACCGA

25 401 AAAGTTTGA ATCAGGCATT TATGTTTTTA GTGAATCCCA AGTTCCAGGA

451 ACAGATGCCT TACAAGCTTT ATATTTAGAT GATCAAGTAA TGAATTTGA

30 501 TTTAACGCCG AATCGTGCAG ATGCTTTAAG TATGATAGGT ACTGCTTATG

551 AAGTTGCAGC ATTATATAAT ACAAAAATGA CTAAGCCAGA GACAACATCA

601 AATGAGCTTG AGTTATCTGC AAATGATGAA TTGACTGTGA CAATTGAAAA

35 651 TGAAGATAAA GTACCATATT ATAGTGCACG TGTTGTTTAC GACGTGACAA

701 TTGAACCTTC GCCAATTTGG ATGCAAGCAC GCTTAATAAA AGCGGGTATA

40 751 CGTCCTATTA ATAATGTTGT TGACATTTCA AATTATGTGT TATTAGAATA

801 CGGTCAACCA TTGCACATGT TTGATCAAGA TGCGATTGGT TCACAACAAA

851 TTGTTGTTTCG TCAAGCTAAT GAAGGCGAAA AAATGACAAC ATTAGATGAT

45 901 ACAGAACGTG AATTATTAAC GAGCGATATT GTCATTACTA ATGGACAAAC



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951 TCCAATTGCA TTAGCTGGTG TTATGGGTGG CGATTTTTC A GAAGTTAAAG  
 1001 AACAAACATC AAATATAGTG ATTGAAGGTG CTATTTTGA TCCAGTTTCA  
 1051 ATTCGTCATA CATCAAGACG TTTAAATT A CGCAGTGAAT CATCTAGTCG  
 1101 TTTTGAAAAA GGAATAGCTA CTGAATTGT AGATGAAGCA GTCGACCGTG  
 1151 CATGTTATTT ATTACAACT TATGCAAACG GAAAAGTGCT AAAAGATAGA  
 1201 GTGTCTTCAG GAGAACTTGG TGCATTTATT ACACCAATCG ACATCACTGC  
 1251 TGATAAAATT AATCGCACTA TTGGATTTGA TTTGTCACAA AATGATATTG  
 1301 TTACTATTTT TAATCAACTA GGGTTTGATA CAGAAATAAA TGATGATGTT  
 1351 ATTACAGTGC TAGTACCATC ACGTCGTAAA GATATTACAA TTAAAGAAGA  
 1401 TTTAATTGAA GAAGTTGCAC GTATATATGG ATACGACGAT ATTCCATCAA  
 1451 CGTTACCTGT CTTGATAAAA GTTACTAGTG GTCAGCTAAC TGATCGCCAA  
 1501 TATAAACTA GAATGGTTAA AGAAGTGTTA GAAGGTGCTG GATTAGATCA  
 1551 AGCTATTACG TATTCGTTAG TTTCTAAAGA GGATGCTACT GCATTTTCGA  
 1601 TGCAACAGCG TCAAACAATT GATTTATTGA TGCCAATGAG TGAAGCGCAT  
 1651 GCGTCATTAC GTCAAAGTTT ATTACCACAT TTAATCGAAG TAGCATCATA  
 1701 TAATGTGGCA CGCAAAAATA AAGATGTAAA ATTATTTGAA ATCGGCAATG  
 1751 TCTTCTTTGC TAATGGAGAA GGTGAACTAC CAGATCAAGT TGAATATTTA  
 1801 AGTGGTATTT TAACTGGAGA TTATGTAGTC AATCAATGGC AAGGTAAGAA  
 1851 AGAAACGGTT GATTTCTATT TAGCAAAAGG TGTCGTGGAT CGAGTATCTG  
 1901 AAAAGTTAAA CCTTGAATTT AGTTATCGTC GTGCTGATAT TGATGGATTA  
 1951 CATCCAGGTC GTECTGCTGA AATCTTATTA GAGAATAAAG TTGTTGGTTT  
 2001 TATTGGTGAA TTACATCCAA CATTAGCAGC TGATAATGAT TTAAACGTA

2051 CGTATGTTTT TGAGTTGAAT TTTGATGCAT TAATGGCTGT GTCGGTAGGT  
 2101 TACATTAATT ACCAGCCAAT TCCGAGATTC CCAGGCATGT CTCGTGACAT  
 5 2151 TSCATTAGAA GTAGATCAAA ATATTCCAGC AGCTGATTTA TTATCAACGA  
 2201 TTCATGCACA CGGTGGCAAT ATATTAAAAG ATACACTTGT CTTTGATGTA  
 2251 TATCAGGGCG AACATTTAGA AAAAGGTAAA AAATCAATTG CAATACGTTT  
 10 2301 AAATTATTTA GACACAGAAG AAACATTGAC AGATGAGCGC GTTTCAAAAG  
 2351 TGCAAGCGGA AATTGAAGCA GCATTAATTG AACAAGGTGC TGTATTAGA

# 15 SEQ ID NO 1C - *S. aureus* Prolyl tRNA Synthetase

1 MKQSKVFIPT MRDVPSEAEA QSHRLLKSG LIQSTSGIY SYLPLATRVL  
 51 NNITAIVRQE MERIDSVEIL MPALQQAELW EESGRWGAYG PELMRLQDRH  
 20 101 GRQFALGPTH EELVTSIVRN ELKSYKQLPM TLFQIQSKFR DEKRPRFGLL  
 151 RGREFIMKDA YSFHADEASL DQTYQDMYQA YSRIFERVGI NARPVVADSG  
 25 201 AIGGSHTHEF MALSAIGEDT IVYSKESDYA ANIEKAEVVY EPNHKHSTVQ  
 251 PLEKIETPNV KTAQELADFL GRPVDEIVKT MIFKVDGEYI MVLVRGHHEI  
 30 301 NDIKLKSYFG TDNIELATQD EIVNLVGANP GSLGPVIDKE IKIYADNFVQ  
 351 DLNNLVVGAN EDGYHLINVN VGRDFNVDEY GDFRFILEGE KLSDGSGVAH  
 401 FAEGIEVGQV FKLGTKYSES MNATFLDNQG KAQPLIMGCY GIGISRTLSA  
 35 451 IVEQNHHDDNG IVWPKSVTPF DLHLISINPK KDDQRELADA LYAEFNTKFD  
 501 VLYDDRQERA GVKFNDADLI GLPLRIVVGK RASEGIVEVK ERLTGDSEEV  
 551 HDDLMTVIT NKYDNLK\*

# 40 SEQ ID NO 2C

1 ATGAAGCAAT CCAAAGTTTT TATACCAACG ATGCGTGATG TGCCATCAGA  
 45 51 AGCAGAAGCA CAAAGTCATC GTTTATTATT GAAATCGGGT TTGATAAAAC

101 AAAGTACAAG TGGGATTTAT AGTTATTTAC CGCTAGCAAC ACGTGTGTTA  
5 151 AATAATATTA CTGCAATTGT GCGACAAGAA ATGGAACGTA TCGATTCTGT  
201 TGAAATTTTA ATGCCAGCGT TACAACAAGC TGAATTATGG GAAGAATCAG  
251 GACGTTGGGG TGCATATGGC CCAGAATTAA TCGGTTTACA AGATAGACAT  
10 301 GGAAGACAAT TTGCATTAGG TCCAACACAT GAAGAATTAG TTACATCAAT  
351 AGTAAGAAAT GAATTGAAAT CATACAAACA ATTACCGATG ACATTATTCC  
15 401 AAATTCAATC TAAATTCCGT GATGAAAAGA GACCACGTTT TGGTTTACTT  
451 CGTGGGCGTG AATTTATTAT GAAAGATGCG TATTCATTCC ATGCTGACGA  
501 GGCATCATTA GATCAAACGT ATCAAGATAT GTATCAAGCG TATAGCCGTA  
20 551 TTTTGTAGAG AGTTGGCATT AATGCTAGAC CAGTTGTAGC AGATTCAGGT  
601 GCTATAGGCG GTAGCCATAC ACATGAATTT ATGGCATTAA GTGCTATCGG  
25 651 TGAGGATACA ATCGTTTACA GTAAAGAAAG TGAATATGCT GCTAATATCG  
701 AAAAAGCAGA AGTCGTTTAC GAACCAAATC ATAAGCATTC TACTGTGCAA  
751 CCTTTAGAAA AAATTGAAAC ACCAAATGTT AAGACTGCAC AAGAATTGGC  
30 801 AGACTTCTTA GGTAGACCAG TAGATGAAAT CGTTAAAACG ATGATTTTCA  
851 AAGTTGATGG CGAATATATT ATGGTTTTAG TCGTGGCCA TCATGAAATT  
901 AATGACATTA AATTAAAATC TTATTTCCGC ACAGATAATA TTGAATTAGC  
35 951 AACACAAGAC GAAATTGTTA ATTTAGTTGG TGCAAATCCG GGTTCACTAG  
1001 GTCCTGTTAT TGATAAAGAA ATCAAAATTT ATGCAGATAA TTTTGTGCAA  
40 1051 GATTTAAATA ATTTAGTTGT CGGTGCTAAC GAAGATGGCT ATCACTTAAT  
1101 TAATGTAAAT GTAGGTAGAG ACTTCAACGT TGATGAATAT GGCGATTTC  
1151 GTTTTATTTT AGAAGGCGAA AAGTTAAGTG ATGGTTCAGG CGTTGCACAT  
45

1201 TTTGCTGAAG GTATTGAAGT TGGTCAAGTA TTCAAATTGG GTACTAAGTA  
 1251 TTCAGAATCA ATGAATGCTA CATTCTTAGA TAACCAAGGA AAAGCTCAAC  
 5 1301 ~~CT~~TTAATTAT GGGCTGTTAC GGTATTGGAA TTTCTAGAAC GCTAAGTGCG  
 1351 ATTGTTGAAC AAAATCACGA TGATAATGGA ATTGTTTGGC CTAAATCAGT  
 1401 TACTCCATTT GATTTCATT TAATTTCTAT TAATCCTAAG AAAGATGATC  
 10 1451 AACGAGAGCT AGCAGATGCA CTATATGCTG ~~AATTTAATAC TAAATTTGAT~~  
 1501 GTGTTGTACG ATGATCGTCA GGAACGTGCA GGTGTCAAAT TTAATGATGC  
 1551 CGATTTAATT GGTTTACCAC TGCGAATTGT TGTGGTAAA CGTGCATCGG  
 1601 AAGGTATTGT AGAAGTTAAA GAACGTTTAA CAGGTGATAG CGAAGAAGTT  
 1651 CACATTGATG ACTTAATGAC TGTCATTACA AATAAATATG ATAACTTAAA  
 20 1701 ATAA

# SEQ ID NO 1D - *S. aureus* Histidyl tRNA Synthetase

25 1 MIKIPRGTD ILPEDSKKWR YIENQLDELM TFYNYKEIRT PIFESTDLFA  
 51 RGVGDSTDVV QKEMYTFKDK GDRSITLRPE GTAAVRSYI EHKMQGNPNQ  
 101 PIKLYYNGPM FRYERKQKGR YRQFNQFGVE AIGAENPSVD AEVLAMVMHI  
 30 151 YQSFGCLKHLK LVINSVGDMA SRKEYNEALV KHFEPIHEF CSDCQSRSLHT  
 201 NPMRILDCKV DRDKEAIKTA PRITDFLNEE SKAYYEQVKA YLDDLGIPIYI  
 35 251 EDPNLVRGLD YYHTAFELM MDNPNYDGAI TTLCGGGRYN GLLELLDGPS  
 301 ETGIGFALSI ERLLLALEEE GIELDIEENL DLFIVTMGDQ ADRYAVKLLN  
 351 HLRHNGIKAD KDYLQRKIKG QMKQADRLGA KFTIVIGDQE LENNKIDVKN  
 40 401 MTTGESETIE LDALVEYFKK \*

# SEQ ID NO 2D

45

1151 TCGTTATTGG TGATCAAGAA TTAGAAAATA ATAAAATCGA TGTTAAAAAT  
 1201 ATGACAACTG GTGAATCTGA AACAAATTGAA TTAGACGCAT TAGTCGAATA  
 1251 TTTTAAGAAG TAG

# SEQ ID NO 1E - *S. aureus* Methionyl tRNA Synthetase

10           1   MAKETFYITT PIYYPSGNLH IGHAYSTVAG DVIARYKRMQ GYDVRYLTGT  
              51   DEHGQKIQEK AQKAGKTEIE YLDEMIAGIK QLWAKLEISN DDFIRTTEER  
              101   HKHVVEQVFE RLLKQGDIYL GEYEGWYSVP DETYYTESQL VDPQYENGKI  
 15           151   IGGKSPDSGH EVELVKEESY FFNISKYTDR LLEFYDQNPD FIQPPSRKNE  
              201   MINNFIKPGL ADLAVSRTSF NWGVHVPSNP KHVYVWIDA LVNYISALGY  
              251   LSDDESLFNK YWPADIHLMA KEIVRFHSII WPILLMALDL PLPKKVFAHG  
              301   WILMKDGKMS KSKGNVDPN ILIDRYGLDA TRYILMRELP FGSDGVFTPE  
              351   AFVERTNFDL ANDLGNLVNR TISMVNKYFD GELPAYQGPL HELDEEMEAM  
 25           401   ALETVKSYTE SMESLQFSVA LSTVWKFISR TNKYIDETTP WVLAKDDSQK  
              451   DMLGNVMAHL VENIRYAAVL LRPFLTHAPK EIFEQLNINN PQFMEFSSLE  
              501   QYGVLTESIM VTGQPKPIFP RLDSEAEIAY IKESMQPPAT EEEKEEIPSK  
 30           551   PQIDIKDFDK VEIKAATIID AEHVKKSDKL LKIQVDLDSE QRQIVSGIAK  
              601   FYTPDDIIGK KVAVVTNLKP AKLMGQKSEG MILSAEKDGV LTLVSLPSAI  
 35           651   PNGAVIK\*

# SEQ ID NO 2E

40           1   ATGGCTAAAG AACATTTTA TATAACAACC CCAATATACT ATCCTAGTGG  
              51   GAATTTACAT ATAGGACATG CATATTCTAC AGTGGCTGGA GATGTTATTG  
              101   CAAGATATAA GAGAATGCAA GGATATGATG TTCGTTATTT GACTGGAACG

151 GATGAACACG GTCAAAAAAT TCAAGAAAAA GCTCAAAAAG CTGGTAAGAC  
 201 AGAAATTGAA TATTTGGATG AGATGATTGC TGAATTAAA CAATTGTGGG  
 5 251 CTAAGCTTGA AATTTCAAAT GATGATTTTA TCAGAACAAC TGAAGAACGT  
 301 CATAAACATG TCGTTGAGCA AGTGTGTTGAA CGTTTATTAA AGCAAGGTGA  
 351 TATCTATTTA GGTGAATATG AAGGTTGGTA TTCTGTTC CG GATGAAACAT  
 10 401 ACTATACAGA GTCACAATTA GTAGACCCAC AATACGAAAA CGGTAAAATT  


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 451 ATTGGTGGCA AAAGTCCAGA TTCTGGACAC GAAGTTGAAC TAGTTAAAGA  
 15 501 AGAAAGTTAT TTCTTTAATA TTAGTAAATA TACAGACCGT TTATTAGAGT  
 551 TCTATGACCA AAATCCAGAT TTTATACAAC CACCATCAAG AAAAAATGAA  
 601 ATGATTAAACA ACTTCATTAA ACCAGGACTT GCTGATTTAG CTGTTTCTCG  
 20 651 TACATCATTT AACTGGGGTG TCCATGTTCC GTCTAATCCA AACATGTTG  
 701 TTTATGTTTG GATTGATGCG TTAGTTAACT ATATTTGAGC ATTAGGCTAT  
 25 751 TTATCAGATG ATGAGTCACT ATTTAACAAA TACTGGCCAG CAGATATTCA  
 801 TTTAATGGCT AAGGAAATTG TGCGATTCCA CTCAATTATT TGGCCTATTT  
 851 TATTGATGGC ATTAGACTTA CCGTTACCTA AAAAAGTCTT TGCACATGGT  
 30 901 TGGATTTTGA TGAAAGATGG AAAAATGAGT AAATCTAAAG GTAATGTTGT  
 951 AGACCCTAAT ATTTTAATTG ATCGCTATGG TTTAGATGCT ACACGTTATT  
 35 1001 ATCTAATGCG TGAATTACCA TTTGGTTCAG ATGGCGTATT TACACCTGAA  
 1051 GCATTTGTTG AGCGTACAAA TTTCGATCTA GCAAATGACT TAGGTAACCT  
 1101 AGTAAACCGT ACGATTTCTA TGGTTAATAA GTACTTTGAT GGC GAATTAC  
 40 1151 CAGCGTATCA AGGTCCACTT CATGAATTAG ATGAAGAAAT GGAAGCTATG  
 1201 GCTTTAGAAA CAGTGAAAAG CTACACTGAA AGCATGGAAA GTTTGCAATT  
 45 1251 TTCTGTGGCA TTATCTACGG TATGGAAGTT TATAAGTAGA ACGAATAAGT

1301 ATATTGACGA AACAAACGCCT TGGGTATTAG CTAAGGACGA TAGCCAAAAA  
 1351 GATATGTTAG GCAATGTAAT GGCTCACTTA GTTGAAAATA TTCGTTATGC  
 1401 AGCTGTATTA TTACGTCCAT TCTTAACACA TGCGCCGAAA GAGATTTTTG  
 1451 AACAAATTGAA CATAAACAAT CCTCAATTTA TGGAATTTAG TAGTTTAGAG  
 1501 CAATATGGTG TGCTTACTGA GTCAATTATG GTTACTGGGC AACCTAAACC  
 1551 TATTTTCCCA AGATTGGATA GCGAAGCGGA AATTGCATAT ATCAAAGAAT  
 1601 CAATGCAACC GCCTGCTACT GAAGAGGAAA AAGAAGAGAT TCCTAGCAAA  
 1651 CCTCAAATTG ATATTAAAGA CTTTGATAAA GTTGAAATTA AGGCAGCAAC  
 1701 GATTATTGAT GCTGAACATG TTAAGAAGTC AGATAAGCTT TTAATAATTC  
 1751 AAGTAGACTT AGATTCTGAA CAAAGACAAA TTGTATCAGG AATTGCCAAA  
 1801 TTCTATACAC CAGATGATAT TATTGGTAAA AAAGTAGCAG TTGTTACTAA  
 1851 CCTGAAACCA GCTAAATTAA TGGGACAAAA ATCTGAAGGT ATGATATTAT  
 1901 CTGCTGAAAA AGATGGTGTA TTAACCTTAG TAAGTTTACC AAGTGCAATT  
 1951 CCAAATGGTG CAGTGATTAA ATAA

30 **SEQ ID NO 1F - *S. aureus* Valyl tRNA Synthetase**

1 MEMKPKYDPR EVEAGRYEEW VKNGYFKPSE DSKETYTIV IPPPNVTGKL  
 51 HLGHAWD TTL QDIITRMKRM QGYDTLYLPG MDHAGIATQA KVEAKLNEQG  
 101 ITRYDLGREK FLEQAWDWKE EYASFIRAQW AKLGLGLDYS RERFTLDEGL  
 151 SKAVKKVFVD LYNKGIIYRG ERIINWDPKA RTALSDIEVI HEDVQGA FYH  
 201 FKYPYADGEG FIEIATTRPE TMLGDTAIVV NPNDERYKDV IGKTVILPIV  
 251 GRELPILADE YVDIDFGSGA MKVTPAHDPN DFEIGQRHQL ENIIIVMDENG  
 301 KMNDKAGKYE GMDRFDCRKQ LVKDLKEQDL VIKIEDHVHS VGHSESRGAV

351 VEPYLSTQWF VRMEDLAKRS LDNQKTDDRI DFYPQRFEHT FNQWMENIRD  
 401 WTISRQLWWG HQIPAWYHKE TGEIYVGEEA PTDIENWQOD EDVLDTWFS  
 5 451 ALWPFSTLGW PDLESEDFKR YYPTNALVTG YDIIFFWVAR MIFQGLEFTD  
 501 RRPFNDVLLH GLVRAEDGRK MSKSLGNGVD PMDVIDEYGA DSLRYFLATG  
 551 SSPGHDLRYS TEKVESVWNF INKIWNGARF SLMNIGEDFK VEDIDLSGNL  
 10 601 SLADKWILTR LNETIATVTD LSDKYEFGEV GRALYNFIWD DFCWDYIEMS  
 651 KIPMNSNDEE QKQVTRSVLS YTLDNIMRML HPFMPFVTEK IWQSLPHEGD  
 15 701 TIVKASWPEV RESLIFEESK QTMQQLVEII KSVRQSRVEV NTPLSKEIPI  
 751 LIQAKDKEIE TTLSQNKDYL IKFCNPSTLN ISTDVEIPEK AMTSVVIAGK  
 801 VVLPLEGLID MDKEISRLEK ELAKLQSELD RVDKKLSNEN FVSKAPEKVI  
 20 851 NEEKRKKQDY QEKYDGVKAR IEQLKA\*

## SEQ ID NO 2F

25 1 ATGGAAATGA AACCAAAATA TGATCCTCGT GAAGTTGAAG CGGGACGTTA  
 51 TGAAGAATGG GTAAAGAATG GTTATTTTAA ACCGTCAGAA GATAAATCAA  
 100 AAGAAACATA TACAATTGTT ATCCCGCCAC CAAATGTAAC TGGTAAATTA  
 30 151 CATTTAGGAC ATGCATGGGA TACGACTTTA CAAGATATCA TTACACGTAT  
 201 GAAACGTATG CAAGGATACG ATACGTTATA CTTACCAGGT ATGGATCATG  
 35 251 CTGGTATTGC GACACAGGCA AAGGTAGAAG CTAAATTAAA TGAACAAGGA  
 301 ATAAC TAGAT ATGATCTTGG TCGTGAAAAG TTTT TAGAAC AGGCATGGGA  
 351 TTGGAAAGAA GAGTATGCGT CATTTATTCG TCGCAATGG GCTAAATTAG  
 40 401 GTCTAGGTTT AGATTATAGT AGAGAACGTT TACTTTAGA TGAAGGTTTA  
 451 AGTAAAGCAG TTAAAAAGT TTTTGTGAT TTATACAATA AAGGAATTAT  
 45 501 TTATCGTGGC GAACGTATTA TAAATTGGGA TCCTAAAGCA CGTACAGCTT



551 TATCTGATAT TGAAGTAATA CATGAAGATG TTCAAGGTGC GTTTTATCAT  
601 TTTAAATATC CTTACGCTGA TGGTGAAGGT TTTATTGAAA TTGCAACAAC  
5 651 AAGACCAGAA ACGATGTTAG GTGATACAGC GATTGTTGTT AACCCTAATG  
701 ACGAACGATA CAAAGATGTA ATCGGTAAAA CTGTTATATT ACCAATCGTA  
10 751 GGACGCGAAC TGCCTATTTT AGCAGATGAG TATGTTGATA TAGACTTCGG  
801 TTCTGGTGCT ATGAAAGTGA CACCAGCACA TGACCCTAAT GATTTTGAAA  
851 TTGGTCAAAG ACATCAATTA GAAAATATTA TCGTTATGGA TGAAAATGGT  
15 901 AAAATGAACG ACAAAGCGGG TAAATATGAA GGTATGGACC GTTTTGATTG  
951 TCGTAAACAG CTAGTTAAAG ATTTAAAAGA ACAAGATTTA GTTATCAAGA  
20 1001 TTGAAGATCA TGTTCAATTCT GTAGGTCATT CAGAACGATC TGGCGCTGTT  
1051 GTTGAACCAT ATTTATCAAC ACAATGGTTT GTGCGCATGG AAGACTTAGC  
1101 GAAACGTTCA TTAGATAACC AAAAAACAGA TGATCGTATT GATTTTTATC  
25 1151 CGCAACGTTT CGAACATACA TTTAACCAAT GGATGGAAAA TATTAGAGAT  
1201 TGGACGATTT CAAGACAATT ATGGTGGGGT CATCAAATTC CGGCTTG GTA  
30 1251 TCATAAAGAA ACAGGCGAAA TATATGTTGG AGAAGAAGCG CCAACTGATA  
1301 TTGAAAATTG GCAACAAGAT GAAGATGTAT TAGATACGTG GTTCTCGAGT  
1351 GCTTTATGGC CTTTCTCTAC GTTAGGTTGG CCTGATTTAG AAAGTGAAGA  
35 1401 CTTTAAACGA TACTACCCAA CAAATGCCTT AGTTACAGGT TACGATATTA  
1451 TCTTTTTCTG GGTCGCACGC ATGATATTCC AAGGCTTAGA ATTTACAGAT  
40 1501 CGTCGTCCAT TTAATGATGT ATTATTAGAC GGTTTAGTTC GTGCTGAAGA  
1551 CGGGCGTAAG ATGAGTAAAT CATTAGGTAA TGGTGTGGAT CCAATGGATG  
1601 TTATTGACGA ATACGGTGCT GATAGCTTGC GTTACTTCTT AGCAACAGGT  
45

1651 TCATCTCCAG GACATGATTT AAGATACTCA ACTGAAAAAG TTGAGTCAGT  
 1701 GTGGAAC TTT ATCAATAAAA TCTGGAATGG GGCACGTTTC AGTTTAATGA  
 5 1751 AFATCGGTGA AGACTTTAAA GTTGAAGATA TCGATTTAAG TGGTAACTTA  
 1801 TCATTAGCAG ATAAATGGAT TCTAACACGT TTAAATGAAA CGATTGCAAC  
 1851 AGTTACTGAT TTAAGTGACA AATATGAATT CGGCGAAGTT GGACGTGCAT  
 10 1901 TATATAATTT CATTG TGGGAT GATTCTCTGTG- ATTGGTACAT-TGAAATGAGT  
 1951 AAAATTCCAA TGAATAGTAA TGATGAAGAA CAAAAACAAG TTACACGTTTC  
 15 2001 AGTATTGAGT TATACTTTAG ACAATATTAT GAGAATGCTA CATCCATTCA  
 2051 TGCCATTTGT AACAGAGAAA ATATGGCAA GTTTACCACA TGAAGGTGAC  
 2101 ACAATTGTTA AAGCTTCATG GCCAGAAGTG CGTGAATCAT TGATTTTTGA  
 20 2151 AGAAAGTAAA CAAACAATGC AACAACTTGT TGAAATCATT AAATCTGTAA  
 2201 GACAATCACG TGTAGAAGTA AATACGCCAT TGTCTAAAGA AATACCTATT  
 25 2251 TTAATTCAAG CTAAAGATAA AGAAATTGAA ACAACTTTAT CACAAAACAA  
 2301 AGATTATTTA ATCAAATTCT GTAATCCTAG TACCTTAAAT ATTAGCACTG  
 2351 ACGTGGAAAT TCCTGAGAAA GCAATGACAT CAGTTGTAAT TGCAGGTAAA  
 30 2401 GTGGTATTAC CATTAGAAGG GCTAATTGAT ATGGATAAGG AAATCAGCCG  
 2451 TTTGGAAAAA GAATTAGCTA AACTTCAAAG CGAATTAGAT AGAGTAGATA  
 35 2501 AAAAGCTCTC TAATGAAAAC TTTGTAAGTA AAGCACCTGA AAAGGTTATA  
 2551 AATGAAGAAA AACGTAAAAA ACAAGATTAT CAAGAAAAAT ATGATGGTGT  
 2601 GAAGGCAAGA ATTGAACAAT TAAAAGCATA G

40

# SEQ ID NO 1G - *S. aureus* Asparaginyl tRNA Synthetase

1 MVMKTTIKQA KDHLNQDVTI GAWLTNKRSS GKIAFLQLRD GTGFMQGVVV  
 45 51 KSEVDEEVFK LAKEIAQESS LYVTGTITED NRSDLGYEMQ VKSIEVISEA

101 HDYPITPKNH GTEFLMDHRH LWLRSKKQHA VMKIRNEVIR ATYEFFNKG  
 5 151 FTKVDPPILT ASAPEGTSEL FHTKYFDQDA FLSQSGQLYL EAAAMAHGKV  
 201 FSPGPTFRAE KSKTRRLHIE FWMIEGEMAF TNHAESLEIQ EQYVTHVVK  
 251 VLENCKLELK ILERDTSKLE KVATPFPRIS YDDAIEFLKA EGFDDIEWGE  
 10 301 DFGAPHETAI ANHYDLPVFI TNYPTKIKPF YMQPNPENEE TVLCADLIAP  
 351 EGYGEIIGGS ERVDDLELLE QRVKEHGLDE EAYSYYDLR RYGSVPHCGF  
 401 GLGLERTVAW ISGVEHVRET APFPRLNLRL YP\*

15  
**SEQ ID NO 2G**

1 ATGGTTATGA AAACAACGAT TAAACAAGCG AAAGATCATT TAAACCAAGA  
 20 51 CGTTACAATT GGTGCTTGGT TAACAAATAA ACGTTCAAGT GGTAAAATCG  
 101 CCTTTTTACA ATTACGTGAT GGAACAGGCT TTATGCAAGG CGTAGTAGTT  
 151 AAATCAGAAG TTGATGAAGA GGTATTCAAA CTTGCGAAAG AAATTGCTCA  
 25 201 AGAATCATCT CTATACGTTA CAGGCACAAT TACAGAAGAT AATCGTTCTG  
 251 ACTTAGGATA CGAAATGCAA GTGAAATCAA TTGAAGTTAT TTCAGAAGCG  
 30 301 CATGACTATC CGATTACACC TAAAAATCAT GGTACAGAAT TCTTAATGGA  
 351 TCACCGTCAT TTATGGTTAC GTTCTAAAAA ACAACATGCT GTAATGAAAA  
 401 TTAGAAATGA AGTTATTCGT GCAACGTATG AATTTTCAA CAAAGATGGA  
 35 451 TTTACAAAGG TTGATCCACC AATTTTGACA GCAAGTGCAC CAGAAGGTAC  
 501 AAGTGAATTA TTCCATACTA AATACTTTGA TCAAGATGCG TTTTATCTC  
 40 551 AAAGTGGTCA GTTATACTTA GAAGCTGGAG CAATGGCACA CGGAAAAGTA  
 601 TTTTCATTTG GTCCAACTTT CAGAGCTGAA AAATCAAAAA CACGTAGACA  
 651 CTTGATCGAG TTCTGGATGA TTGAAGGGGA AATGGCTTTC ACAAATCATG

45

701 CTGAAAGTTT AGAAATTCAA GAACAATATG TAACACATGT AGTAAATCA  
 751 GTTTTAGAAA ATTGTAAACT AGAGTTGAAA ATTTTAGAGC GTGATACATC  
 5 801 ~~AAAACTTGAA~~ AAAGTTGCGA CACCATTCCC TAGAATTTCA TATGATGATG  
 851 CAATTGAATT CTTAAAAGCA GAAGGCTTTG ATGATATTGA ATGGGGTGAA  
 901 GATTTTGGTG CGCCACATGA AACTGCCATT GCTAATCATT ATGATTACC  
 10 951 GGTGTTTATT ACTAATTATC CAACTAAAAT TAAGCCTTTC TATATGCAAC  
 -----  
 1001 CAAATCCTGA GAATGAAGAA ACTGTCTTAT GTGCAGACTT AATTGCACCT  
 15 1051 GAAGGATACG GTGAAATTAT CGGTGGATCT GAACGTGTGG ATGACTTAGA  
 1101 ATTGTTAGAA CAACGCGTTA AAGAACATGG ATTAGACGAA GAAGCATATA  
 1151 GTTACTACTT AGACTTACGT CGTTATGGTA GTGTGCCACA CTGTGGATTT  
 20 1201 GGTTTAGGTT TAGAGCGCAC AGTAGCATGG ATTTCTGGTG TTGAACACGT  
 1251 TCGTGAAACT GCGCCATTCC CAAGATTATT AAACCGTTTA TATCCATAA

25 **SEQ ID NO 1H - *S. aureus* ArgininyI tRNA Synthetase**

1 MNIIDQVKQT LVEEIAASIN KAGLADEIPD IKIEVPKDTK NGDYATNIAM  
 51 VLTAKIARNP REIAQAIVDN LDTEKAHVQK IDIAGPGFIN FYLDNQYLTA  
 30 101 IIPAEIEKGD QFGHVNESKG QNVLLLEYVSA NPTGDLHIGH ARNAAVGDAL  
 151 ANILTAAGYN VTREYYINDA GNQITNLARS IETRFEEALG DNSYSMPEDG  
 35 201 YNGKDIIEIG KDLAEKHPEI KDYSEEARLK EFRKLGVEYE MAKLKNDLAE  
 251 FNTHFDNWFS ETSLYEKGEI LEVLAKMKEL GYTYEADGAT WLRTTDFKDD  
 301 KDRVLIKNDG TYTYFLPDIA YHFDKVKRGN DILIDLFGAD HHGYINRLKA  
 40 351 SLETFGVDSN RLEIQIMQMV RLMENGKEVK MSKRTGNAIT LREIMDEGVV  
 401 DAARYFLTMR SPDSHFDFDM ELAKEQSQDN PVYYAQYAHA RICSILKQAK  
 45 451 EQGIEVTAAN DFTTITNEKA IELLKKVADF EPTIESAAEH RSAHRITNYI

501 QDLAAHFHKF YNAEKVLDD IEKTKAHVAM IEAVRITLKN ALAMVGV SAP

551 ESM\*

# SEQ ID NO 2H

1 ATGAATATTA TTGATCAAGT GAAACAAACA TTAGTAGAAG AAATTGCAGC

51 AAGTATTAAC AAAGCAGGAT TAGCAGATGA GATTCCTGAT ATTAAAATTG

101 AAGTTCCTAA AGATACAAAA AATGGAGATT ATGCTACTAA TATTGCGATG

151 GTACTGACTA AGATTGCAAA GCGTAATCCT CGTGAAATTG CTCAAGCGAT

201 TGTTGATAAC TTAGATACTG AAAAAGCACA TGTAACAA ATTGACATTG

251 CTGGTCCAGG ATTCATTAAT TTTTACTTAG ATAATCAGTA TTTAACAGCA

301 ATTATTCCTG AAGCAATTGA AAAAGGTGAT CAATTTGGAC ATGTAAATGA

351 ATCAAAAGGT CAAAATGTAT TGCTTGAGTA TGTTTCAGCT AACCCCTACAG

401 GAGATTTACA TATTGGTCAT GCTAGAAATG CAGCAGTTGG TGATGCTTTA

451 GCTAATATTT TAACTGCAGC TGGCTATAAT GTAACACGTG AATATTATAT

501 TAATGATGCT GGTAATCAAA TTTACTAATT AGCGCGTTCG ATTGAAACAC

551 GTTTCCTTGA AGCTTTAGGT GACAATAGTT ATTCAATGCC AGAAGATGGC

601 TATAATGGAA AAGATATTAT TGAAATAGGT AAAGATTTAG CAGAGAAACA

651 CCCTGAAATT AAAGATTATT CTGAAGAAGC ACGTTTGAAA GAATTTAGAA

701 AATTAGGCGT AGAATACGAA ATGGCTAAAT TGAAAAATGA TTTAGCAGAG

751 TTCAATACGC ATTTTGATAA TTGGTTTAGT GAAACATCTT TATATGAAAA

801 AGGCGAAATT CTTGAAGTTT TAGCAAAAT GAAAGAATTA GGTTATACGT

851 ATGAAGCTGA TGGCGCTACA TGGTTACGTA CAACTGATTT TAAAGACGAC

901 AAAGACAGAG TATTAATTAA AAATGACGGT ACATATACGT ATTTCTTACC

951 AGATATTGCG TACCACTTCG ATAAAGTTAA ACGTGGTAAT GACATTTTAA  
1001 TCGATTTATT TGGTGCTGAT CATCATGGTT ATATTAATCG TTTGAAAGCA  
5 1051 TCTCTTGAAA CGTTTGGTGT AGATAGTAAT CGTTTAGAAA TTCAAATCAT  
1101 GCAAATGGTT CGTTTAATGG AAAATGGTAA AGAAGTGAAG ATGAGTAAAC  
1151 GTACTGGTAA TCGGATTACA TTAAGAGAAA TTATGGACGA AGTTGGCGTT  
10 1201 GACGCTGCAC GTTATTTCTT AACTATGCGT AGTCCTGATA GTCACCTTGA  
1251 TTTTGATATG GAATTAGCGA AAGAGCAATC TCAAGACAAT CCAGTTTACT  
15 1301 ATGCTCAATA TGCACATGCG CGTATTTGTT CAATTTTAAA ACAAGCGAAA  
1351 GAGCAAGGTA TTGAAGTGAC TGCTGCGAAT GATTTTACAA CGATTACAAA  
1401 TGAAAAAGCG ATTGAATTGT TGAAAAAAGT AGCTGATTTC GAACCTACAA  
20 1451 TTGAAAGTGC TGCTGAGCAT AGATCAGCAC ATAGAATTAC TAACTATATT  
1501 CAAGACTTGG CTGCTCATTT CCATAAATTC TATAATGCTG AAAAAGTGTT  
25 1551 AACAGATGAT ATTGAAAAAA CAAAAGCACA TGTGCTATG ATTGAAGCGG  
1601 TCAGAATTAC ATTGAAAAAT GCATTGGCAA TGGTCGGTGT AAGCGCACCT  
1651 GAATCAATGT AA  
30

**Claims**

- 5 1. A polypeptide isolated from *S.aureus* WCUH29, characterised in that it comprises an amino acid sequence selected from SEQ ID NO 1A, SEQ ID NO 1B, SEQ ID NO 1C, SEQ ID NO 1D, SEQ ID NO 1E, SEQ ID NO 1F, SEQ ID NO 1G and SEQ ID NO 1H, or a fragment, analogue or derivative thereof.
- 10 2. The polypeptide having the amino acid sequence selected from SEQ ID NO 1A, SEQ ID NO 1B, SEQ ID NO 1C, SEQ ID NO 1D, SEQ ID NO 1E, SEQ ID NO 1F, SEQ ID NO 1G and SEQ ID NO 1H or a derivative thereof.
3. A polynucleotide (DNA or RNA) which encodes a polypeptide according to claim 1.
- 15 4. A polynucleotide having the DNA sequence selected from SEQ ID NO 2A, SEQ ID NO 2B, SEQ ID NO 2C, SEQ ID NO 2D, SEQ ID NO 2E, SEQ ID NO 2F, SEQ ID NO 2G and SEQ ID NO 2H.
- 20 5. A polynucleotide from *S.aureus* WCUH29 characterised in that it comprises the DNA sequence selected from SEQ ID NO 2A, SEQ ID NO 2B, SEQ ID NO 2C, SEQ ID NO 2D, SEQ ID NO 2E, SEQ ID NO 2F, SEQ ID NO 2G and SEQ ID NO 2H.
- 25 6. A protein obtainable by expressing the polynucleotide having the DNA sequence SEQ ID NO 2A, SEQ ID NO 2B, SEQ ID NO 2C, SEQ ID NO 2D, SEQ ID NO 2E, SEQ ID NO 2F, SEQ ID NO 2G and SEQ ID NO 2H.
7. An inhibitor of the polypeptide according to claim 1 or 2.
- 30 8. A pharmaceutical composition comprising an inhibitor as claimed in claim 7 and a pharmaceutically acceptable carrier or excipient.
9. The use of an inhibitor according to claim 7 in therapy.
- 35 10. A method of screening drugs to identify those which interfere with the interaction of a tRNA synthetase which method comprises measurement of enzyme activity by the full aminoacylation reaction or the partial PPi/ATP exchange reaction.

**SB**  
**SmithKline Beecham**  
*Pharmaceuticals*

Attorney Docket No.: P31353

Applicant: Lawlor, et al.

Serial No.: 08/785,455 Filed: January 17, 1997

For: Novel tRNA Synthetase